

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 9/00, A23K 1/165, 1/18</b>		<b>A1</b>	(11) International Publication Number: <b>WO 95/22601</b>
			(43) International Publication Date: <b>24 August 1995 (24.08.95)</b>
(21) International Application Number: <b>PCT/GB95/00336</b>		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).	
(22) International Filing Date: <b>17 February 1995 (17.02.95)</b>			
(30) Priority Data: <b>9403106.9 18 February 1994 (18.02.94) GB</b>			
(71) Applicant (for all designated States except US): THE MINISTER OF AGRICULTURE, FISHERIES AND FOOD IN HER BRITANNIC MAJESTY'S GOVERNMENT OF THE UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND [GB/GB]; Whitehall, London SW1A 2HH (GB).		Published With international search report.	
(72) Inventor; and (75) Inventor/Applicant (for US only): PUGH, Ronald [GB/GB]; 19 Montpelier, Quarndon, Derby, Derbyshire DE22 5JW (GB).			
(74) Agent: LOCKWOOD, Peter, Brian; Ministry of Defence, Directorate of Intellectual Property Rights, Room 2002, Empress State Building, Lillie Road, London SW6 1TR (GB).			
(54) Title: POULTRY FOODSTUFF ENZYMES			
(57) Abstract			
<p>A composition for improving digestibility of leguminous foodstuffs by poultry is provided comprising cellulase, acid or alkaline protease, alpha-galactosidase and zylanase and/or amylase. Preferred compositions have proportions of enzymes therein of 20-40 % wt cellulase; 20-40 % acid or alkaline protease; 20-40 % alpha-galactosidase and 5 to 15 % zylanase and/or amylase. Feedstuffs are provided comprising the enzyme compositions together with seeds, preferably peas, beans, rapeseed and soyabean meal. Preferred feedstuffs have the activity of the enzymes in units of 1200 - 2000 CMCase per gramme and 2000 - 4000 CI ase per gramme of cellulase, 400 - 800 protease per gramme, 400 - 800 GA per gramme alpha galactosidase and 200 - 300 FA per gramme amylase.</p>			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

POULTRY FOODSTUFF ENZYMES.

The present invention relates to compositions comprising enzymes for use in potentiating the nutritive value of foodstuffs, particularly foodstuffs for poultry feeds. These compositions have utility as foodstuff additives whereby they improve digestibility.

Approximately 650 million broiler birds are grown annually in the UK and of these approximately 400 million are fed on feeds including leguminous material. Crop produce such as whole rape, beans, peas and soya bean meal are widely used in poultry diets with inclusion rates in total being as high as 25%. These and other such feedstuff materials contain non-starch polysaccharides (NSPs), including viscous polysaccharides present in wheat and barley, which lead to their inefficient digestion by the subject birds. Such NSPs are thus referred to as anti-nutritive factors and potentially have harmful effects. Beta-glucanases have been incorporated into high barley diets and pentosanases in high wheat diets to try to alleviate this problem with some degree of success.

Known available commercial enzyme cocktails have been analysed as containing activities such as those of beta-glucanase, CM-cellulase, Filter-Paperase, Cellobiase, Xylanase, alpha-Amylase, Protease and alpha-Galactosidase (see International Milling Flour and Feed (1991), 184(7), p33-36.

Leguminous seeds and rape seeds also contain significant quantities of anti-nutritive factors such as lignin, tannins, lectins and anti-trypsin factors in addition to high levels of NSPs. These seeds also contain oligosaccharides such as raffinose and stachylose, while rape seeds also contain fairly high levels of lignins and cellulose which are poorly digested by poultry. It is thus desirable that such mixtures of whole rape seeds, leguminous seeds such as peas, field beans and soya beans are combined with a composition that would render

their digestion by poultry easier.

The present inventor has now provided a composition comprising a mixture of enzymes that he has determined improves the digestibility of leguminous feeds such that improved liveweight gain and feed conversion is provided; feedstuffs incorporating these, particularly those based upon rapeseed, leguminous materials and particularly the commercially important soyabean meal feed, are also provided.

Thus in a first aspect of the present invention there is provided a composition comprising cellulase, acid or alkaline protease, alpha-galactosidase and zylanase and/or amylase. Preferably the composition also comprises amylases.

Preferred proportions of the enzymes in the mixture are 20-40% cellulase; 20-40% acid or alkaline protease; 20-40% alpha-galactosidase and 5 to 15% zylanase and/or amylase. Such composition may be in turn admixed with amylases or these may be present in the individual component enzymes as supplied by commercial manufacturers.

More preferred proportions of the enzymes are about 30% cellulase, about 30% acid or alkaline protease, about 30% alpha-galactosidase and about 10% zylanases. It will be appreciated by those skilled in the art that commercial sources of such enzymes are commonly in the form of 'cocktails' containing a variety of enzymes having the stated activity. Suitable enzymes are available from eg. Alltech, UK.

For increasing the assimilation of soyabean meal feedstuffs a preferred enzyme composition comprises 35% cellulase, 35% protease, 25% alpha-galactosidase and 5% amylase. Such enzyme mixture is admixed with the soybean meal to provide the necessary units per gramme feedstuff described above.

In a second aspect of the invention there is provided a feedstuff

comprising leguminous plant material, preferably including seeds, preferably peas, beans, rapeseed and soyabean meal, together with a composition as described above.

The activity of the enzymes in a preferred mixture including amylase rather than zylanase is as follows:

Cellulase:	1200 - 2000 CMCase	per gramme
	2000 - 4000 CI ase	" "
Protease:	400 - 800 Protease	" "
Alpha-galactosidase:	400 - 800 GA	" "
Amylase:	200 - 300 FA	" "

Use of the feedstuffs of the present invention has been shown to provide a comparatively cheap source of metabolisable energy; cheaper than feed fats in the M.E. range 12.8 to 13.5 MJ/Kg; improved feed conversion rates for broilers and turkeys, better litter quality thus improved bird welfare, improved product quality and less downgrading.

The compositions and feedstuffs of the invention and their use will now be described by way of illustration only by reference to the following non-limiting Examples.

#### EXAMPLE 1:

Feedstuff comprising a composition of the invention at the following activities were fed to groups of 8 birds and the wt, excreta, and gross energy were calculated. Enzymes were from Alltech, UK. This mix is referred to as ALLTECH in the tables below.

Enzyme activity:

Cellulase:	1600 CMCase	per gramme
	3000 CI ase	" "
Protease:	600 Protease	" "
Alpha-galactosidase:	600 GA	" "
Amylase:	250 FA	" "

Results are shown in Tables 1 to 7 below:

TABLE 1: Glucose control					
Bird	Excreta	Gross Energy	N(g/Kg)	EEL(Kj)	EELn(Kj)
2	6.04 g	13.00 KJ/g	216.10	78.52	33.62
7	6.41	13.30	111.00	85.25	60.78
12	5.85	13.65	225.40	79.85	34.49
15	5.63	14.60	161.30	82.20	50.96
19	4.17	14.77	113.00	61.59	45.38
24	6.12	13.52	209.60	82.74	38.62
27	6.02	13.35	195.20	80.37	39.94
30	4.07	14.78	169.50	60.15	36.42

TABLE 2: Foodstuff: HP SOYA control					
Bird	Excreta	Gross Energy	N(g/Kg)	TME(Kj)	TME <sub>n</sub> (Kj)
1	7.33 g	13.88 KJ/g	108.20	14.89	11.58
8	6.52	13.78	106.10	16.08	12.42
9	7.47	13.96	112.50	14.64	11.49
16	6.34	13.83	144.30	16.30	13.41
18	7.40	13.95	128.90	14.74	11.99
23	7.83	13.21	148.70	14.72	12.69
25	9.42	13.45	150.70	12.39	11.24
32	8.16	14.84	116.30	12.95	10.18

TABLE 3: Foodstuff: PURA 42 control					
Bird	Excreta	Gross Energy	N(g/Kg)	TME(Kj)	TME <sub>n</sub> (Kj)
3	8.34 g	14.68 KJ/g	106.00	16.91	15.39
5	8.69	14.77	127.80	16.32	15.58
10	10.02	14.51	127.50	14.61	14.45
14	9.28	14.81	157.80	15.41	15.89
17	9.19	15.46	134.20	14.95	14.63
22	8.23	14.86	110.80	16.92	15.50
28	8.44	15.58	101.00	16.00	14.38
29	7.21	15.52	115.10	17.96	16.26

TABLE 4: Foodstuff: EXTROPRO control					
Bird	Excreta	Gross Energy	N(g/Kg)	TME(Kj)	TME <sub>n</sub> (Kj)
4	9.99 g	14.82 KJ/g	126.70	14.37	14.04
6	10.28	15.23	146.60	13.52	14.02
11	10.02	14.97	176.20	14.17	15.56
13	10.28	14.78	165.30	13.98	15.14
20	9.67	15.41	165.70	14.27	15.10
21	8.24	14.68	162.70	17.08	17.00
26	8.32	14.23	130.90	17.33	16.40
31	9.41	15.97	141.50	14.15	14.04

TABLE 5: Foodstuff: HP SOYA + 2KG/T ALLTECH mix					
Bird	Excreta	Gross Energy	N(g/Kg)	TME(Kj)	TME <sub>n</sub> (Kj)
34	9.33 g	13.43 KJ/g	158.80	12.53	11.59
36	9.39	13.23	181.00	12.64	12.54
41	7.51	13.47	182.90	14.95	13.64
42	9.61	13.09	180.30	12.48	12.41
47	9.22	14.25	157.50	11.92	10.88
49	9.22	13.07	166.00	13.01	12.24
53	8.95	13.34	170.00	13.12	12.32
55	7.62	13.88	188.30	14.49	13.39

TABLE 6: Foodstuff: PURA 42+2KG/T ALLTECH					
Bird	Excreta	Gross Energy	N(g/Kg)	TME(Kj)	TME <sub>n</sub> (Kj)
35	8.65 g	14.71 KJ/g	151.50	16.43	16.38
37	7.63	15.82	139.60	17.08	16.19
40	7.31	15.30	184.80	17.97	18.06
44	7.95	14.11	170.00	17.94	18.02
45	7.03	14.66	132.40	18.85	17.49
50	7.97	14.61	159.90	17.51	17.33
52	7.16	14.33	161.10	18.89	18.30
54	7.39	14.47	152.60	18.46	17.78



TABLE 7: Foodstuff: EXTRUPRO + 2KG/T ALLTECH					
Bird	Excreta	Gross Energy	N(g/Kg)	TME(Kj)	TME <sub>n</sub> (Kj)
33	7.56 g	15.25 KJ/g	135.00	17.64	16.47
38	9.35	14.23	147.10	15.87	15.92
39	8.98	13.95	148.80	16.65	16.56
43	8.90	14.60	136.10	16.18	15.66
46	8.56	14.38	160.60	16.86	16.91
48	6.76	14.22	117.00	19.56	17.60
51	8.55	13.64	156.40	17.51	17.43
56	6.90	15.92	102.90	18.19	15.95

The input values of the foodstuffs in the Tables 1 to 7 are as follows:

Foodstuff:	Input (g)	N(g)/Kg	GE(KJ/g)	DM(g/Kg)
HP SOYA	10.00	77.20	17.43	866.00
PURA 42	10.00	34.30	21.52	905.40
EXTRUPRO	10.00	37.90	21.54	923.00
HP SOYA+2KG/T ALLTECH	10.00	77.20	17.43	866.00
PURA 42+2KG/T ALLTECH	10.00	34.30	21.52	905.40
EXTRUPRO+2KG/T ALLTECH	10.00	37.90	21.54	923.00

The results show an average increase in KJ/g of 4% with the SOYA ALLTECH mixture, 14% with the PURA 42 ALLTECH mixture and 9% with the EXTRUPRO ALLTECH mixture based upon their respective controls.

CLAIMS.

1. A composition comprising cellulase, acid or alkaline protease and alpha-galactosidase together with zylanase and/or amylase.
2. A composition as claimed in claim 1 comprising amylases.
3. A composition as claimed in claim 1 or 2 wherein the proportions of enzymes therein are 20-40% wt cellulase; 20-40% acid or alkaline protease; 20-40% alpha-galactosidase and 5 to 15% zylanase and/or amylase.
4. A composition as claimed in claim 3 comprising about 30% cellulase, about 30% acid or alkaline protease, about 30% alpha-galactosidase and about 10% zylanase or amylase.
5. A composition as claimed in claim 4 wherein the activity of the enzymes in units is 1200 - 2000 CMCase cellulase per gramme, 2000 - 4000 CIase cellulase per gramme, 400 - 800 protease per gramme, 400 - 800 GA per gramme alpha galactosidase and 200 - 300 FA per gramme amylase.
6. A composition as claimed in claim 5 wherein the activity of the enzymes in units is 1600 CMCase per gramme and 3000 CIase per gramme; 600 per gramme protease, 600 GA per gramme alpha-galactosidase and 250 FA per gramme amylase.
7. A feedstuff comprising leguminous plant material, admixed with a composition as claimed in any one of claims 1 to 5.
8. A feedstuff as claimed in claim 6 including seeds, preferably peas, beans, rapeseed and soyabean meal, together with a composition as described above.
9. A feedstuff as claimed in claim 7 comprising about 2Kg of enzyme composition of any one of claims 1 to 6 per tonne.

10. A feedstuff as claimed in any one of claims 7 to 9 wherein the feedstuff comprises soyabeal meal.

11. A method of farming poultry comprising feeding birds with a feedstuff as claimed in claim 8 or 9.

12. A poultry product comprising a bird or part thereof, the bird being one fed with a feedstuff as claimed in claim 8 or 9.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 95/00336

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12N9/00 A23K1/165 A23K1/18

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 8633, Derwent Publications Ltd., London, GB; AN 86-216609 & JP,A,61 149 075 (TAIYO SANGYO KK) 7 July 1986 see abstract ---	1,2
X	JOURNAL OF FERMENTATION AND BIOENGINEERING, vol.69, no.1, 1990, JP pages 8 - 15 CHITTRA MISHRA ET AL. 'Recovery and fractionation of the extracellular degradative enzymes from Lentinula edodes cultures cultivated on a solid lignocellulosic substrate' see page 9, column 2; table 1 ---	1
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

20 April 1995

Date of mailing of the international search report

04. 05. 95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Td. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Dekeirel, M

# INTERNATIONAL SEARCH REPORT

Intern. Appl. No.  
PCT/GB 95/00336

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INTERNATIONAL MILLING FLOUR AND FEED, vol.184, no.7, 1991, UK pages 33 - 36 HADDEN GRAHAM 'Enzymes in monogastric feeds' cited in the application see page 34, column 3; table 5 ---	1,2
Y	PROCEEDINGS, 19th WORLD'S POULTRY CONGRESS, AMSTERDAM, NL, 20-24 September 1992 WORLD'S POULTRY SCIENCE ASSOCIATION, 1992, pages 241-245 B.A. SLOMINSKI ET AL. 'Enhancement of the feeding value of low-glucosinate rapeseed by the supplementation of poultry diets with exogenous enzymes' see the whole document ---	1,7,11, 12
A	---	8,10
Y	DD,A,296 407 (INDUSTRIEFORSCHUNGSZENTRUM BIOTECHNOLOGIE) 5 December 1991 see the whole document ---	1,7,11, 12
A	---	2
A	FEED COMPOUNDER, vol.13, no.1, 1993, UK pages 19 - 21 CLIFFORD A. ADAMS ET AL. 'Non-starch polysaccharides and their digestion in poultry' see the whole document ---	1-12
A	EP,A,0 257 996 (SUOMEN SOKERI OY) 2 March 1988 see page 2, line 62 - page 3, line 5 see example 4 ---	1,2
A	DATABASE WPI Week 9503, Derwent Publications Ltd., London, GB; AN 95-020254 & SU,A,2 007 449 (ALMA-ATA KAZA INVENTORS SOVET) 15 February 1994 see abstract ---	1,2
A	DATABASE WPI Week 8523, Derwent Publications Ltd., London, GB; AN 85-138857 & JP,A,60 075 238 (TSUBAKI T) 27 April 1985 see abstract ---	1,2

-/--

# INTERNATIONAL SEARCH REPORT

Intern. Appl. No.

PCT/GB 95/00336

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO,A,91 04673 (NOVO NORDISK) 18 April 1991  see page 3, line 21 - line 26  see claims 1-5</p> <p>-----</p>	1

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/GB 95/00336

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DD-A-296407		NONE	
EP-A-0257996	02-03-88	JP-A- 63157938 NO-B- 175033	30-06-88 16-05-94
WO-A-9104673	18-04-91	DE-D- 69007115 DE-T- 69007115 EP-A- 0494916 JP-T- 5500807	07-04-94 09-06-94 22-07-92 18-02-93